NEOGEN
VERATOX DON 2/3

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GENERAL INFORMATION

VERATOX DON 2/3 is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) which allows the user to determine the concentration of deoxynivalenol (DON) in parts per million (ppm). Free DON in the samples and controls is allowed to compete with enzyme-labeled DON (conjugate) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate to product blue color. More blue color means less DON. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of DON.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	*Neogen Corporation 800/234-5333*
Test Kit Name:	VERATOX DON 2/3
Product Number:	8335
Effective Date of Instructions:	03/17/2015
Instructions Revision Number	0
Conformance Range:	0.5 – 5.0 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Wheat, corn, barley, corn bran, corn germ meal, corn gluten meal, corn grits, malted barley, malted barley flour, oats, rice, raw rye, rye flour, wheat bran, wheat bran aleurone, wheat flour, wheat flour 2nd clear, and wheat middlings
Extraction method:	Shake vigorously 50-gram sample with 250 mL deionized water for 3 minutes.
Test Format:	Competitive direct enzyme-linked immunosorbent assay
Detection Method:	Stat Fax Reader, Model 321 Plus, Stat Fax Reader, Model 4701

PREPARATION OF TESTING MATERIALS

Stat Fax 321 set up:

(1) Turn reader on and wait for the screen to say Ready.

(2) Press Menu.

(3) Select the Veratox DON test by pressing the corresponding number on the keypad and press Enter.

(4) Press 9 and enter then number of wells that will be tested and press Enter.

(5) Press Enter to start reading the wells when ready.

Stat Fax 4701 set up:

(1) Turn reader on and wait for the main menu to appear.

(2) Press Run Test.

(3) Select the DON test from the menu.

(4) Select "Yes" to accept that test.

(5) Press # Wells and enter how many wells will be read then press OK.

(6) Press Accept then Start when the wells are ready to be read.

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

The sample to be tested should be collected according to accepted sampling technique

(1) Obtain a representative sample

(2) Grind the sample so that at least 95% of the ground material passes through a 20 mesh sieve, about the particle size of fine instant coffee.

Standard Extraction Procedure:

(1) Weigh 50 ± 0.2 grams ground samples into a suitable container or bag.

(2) Add 250 mL of distilled or deionized water and close the bag securely to prevent spillage.

(3) Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for three minutes.

(4) Let the extract sit for 3 minutes to allow for some of the particles to settle.

(5) Filter about 3-5 mL of the extract through a Neogen syringe filter.

(6) For Corn Germ Meal and Malted Barley, check the pH of the filtered extract. For all other commodities, proceed to step 7.

a. If the pH is not between 7.0 -8.0 it needs to be adjusted.

b. Using a disposable polyethylene transfer pipette, add one drop of 1N NaOH (sodium hydroxide) to the sample extract, vortex to mix, and check the pH.

c. If pH is still below 7.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until the pH falls between 7.0 and 8.0, and then proceed to step 7.

(7) Dilute the sample extract 1 to 1 with distilled or deionized water. For example, add 1 mL of extract to 1 mL of distilled or deionized water. Vortex for 10 seconds.

(8) This is the diluted filtered extracted and ready for testing. The diluted filtered extract can be used for next 4 hours.

TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents and antibody wells to reach room temperature (68 - 77ºF) prior to running the test.

(2) Remove 1 red-marked mixing well for each sample to be tested plus 5 red-marked wells for controls and place in the well holder.

(3) Remove an equal number of antibody-coated wells. Return antibody wells which will not be used immediately to the foil pack with desiccant and reseal to protect the antibody. Mark one end of strip with a "1" and place strip in the well holder with the marked end on the left. Do not mark the inside or bottom of the wells.

(4) Mix each reagent by swirling the reagent bottle prior to use.

(5) Place 100 µL of conjugate from the blue-labeled bottle in each red-marked mixing well.

(6) Using a new pipette tip for each, transfer 100 µL of controls and samples to the red-marked mixing wells.

(7) Using a 12-channel pipettor, mix the liquid in the wells by pipetting it up and down 3 times. Transfer 100 µL into the antibody-coated wells.

(8) Mix by sliding the microwell holder back and forth on a flat surface for 10-20 seconds without splashing the reagents from the wells. Incubate for 2 minutes at room temperature (10 – 30ºC, 64 – 86ºF). Discard red-marked mixing wells.

(9) Shake out the contents of the antibody wells. Fill the wells with distilled or deionized water and dump them out. Repeat this step 5 times, then turn the wells upside-down and tap out on a paper towel until the remaining water has been removed.

(10) Pour the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat.

(11) With new tips on the 12-channel pipettor, prime and pipette 100 µL of substrate into the wells and mix by sliding back and forth on a flat surface for 10-20 seconds.

(12) Incubate for 3 minutes. Discard remaining substrate and rinse the reagent boat with water.

(13) Pour Red Stop solution from the red-labeled bottle (same volume as the substrate) into the red-labeled reagent boat.

(14) Using 12-channel pipettor, prime the tips, and pipette 100 µL of Red Stop to each well. Mix by sliding back and forth on a flat surface. Discard the tips.

(15) Wipe the bottom of the microwells with a lint free Kim wipe and read on the Stat Fax reader using a 650 nm filter. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within 20 minutes after the addition of Red Stop.

SUPPLEMENTAL ANALYSIS

Supplemental analysis (for wheat and corn only) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation. The range for performance evaluation of quantitative DON test kits is 0.5 – 5.0 ppm. Therefore, supplemental analysis would be performed for a result above 5.0 ppm. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 0.5 -5.0 ppm), and a correction for dilution is applied to derive at the final result. Supplemental analysis is performed only at the request of the applicant.

a. <u>Supplemental Analysis Procedure</u>:

(1) Dilute the diluted filtered extract that tested above 5 ppm in distilled or deionized water. In a separate tube combine 1 mL of the diluted filtered extract with 1 mL of distilled or deionized water for a dilution factor of 2.

(2) Vortex for 10 seconds and proceed to the test procedure

b. <u>Test Procedure to analyze the samples</u>

(1.) Follow the identical test procedure described above under "**TEST PROCEDURE**".

c. Interpreting Results

Read test results and multiply all results by 2 (two) to calculate the actual value for a 1 to 1 dilution.

Example: Stat Fax results: 4.0 ppm
Times dilution factor: x 2
TOTAL: **8.0 ppm**

A final result less than 3.5 ppm using the supplemental analysis is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the sample extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 5.0 ppm.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

The kit can be used until the expiration date on the label when stored refrigerated at 2-8°C (35-46°F)

b. Precautions

(1) Store test kit between 2-8°C (35-46°F) when not in use, do not freeze.

(2) Do not use kit components beyond expiration date.

(3) Do not mix reagents from one kit serial with reagents from a different kit serial.

(4) Do not run more than 24 wells per test.

(5) Follow proper pipetting techniques, including priming pipette tips by filling and dispensing solution once before use.

(6) Use of incubation times other than those specified may give inaccurate results

(7) Bring kits to room temperature (18-30°C, 64-86°F) prior to use.

(8) Avoid prolonged storage of kits at ambient temperatures.

(9) Treat all used liquids, including sample extract, and labware as if contaminated with DON. Use precaution when handling

(10) To avoid cross-contamination, use new pipette tips for each sample.

(11) Commodities tested should have a pH of 6-8. Excessively acidic of alkaline samples should be adjusted. For instructions on adjusting pH contact your Neogen representative or Technical Services.

(12) Do not use substrate that has turned blue prior to use.

EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits (24 well kit).

(1) 48 antibody-coated microwells

(2) 48 red-marked mixing wells

(3) 5 yellow-labeled bottles of 0, 0.5, 1, 2, and 6 ppm DON controls

(4) 1 blue-labeled bottle of DON-HRP conjugate solution

(5) 1 green-labeled bottle of K-Blue Substrate solution

(6) 1 red-labeled bottle of Red Stop solution

(7) Directions for use

b. Materials required but not provided.

(1) Extraction materials
- i. Distilled or deionized water
- ii. Graduated cylinder
- iii. Container with 500 mL capacity for GIPSA method.
- iv. Neogen filter syringe, Whatman #1 filter paper, or equivalent (Neogen item #9420/#9430)
- v. Sample collection tubes (Neogen item #9421)

(2) 250 mL graduated cylinder (Neogen item #9447)

(3) Agri-Grind grinder or equivalent (Neogen item #9401)

(4) Scale capable of weighing 5-25 grams (Neogen item #9427)

(5) 100 µL pipettor (Neogen item #9272/#9278)

(6) 12-channel pipettor (Neogen item #9273)

(7) Tips for 12-channel and 100 µL pipettors (Neogen item #9410/#9407)

(8) Paper towels or equivalent absorbent material

(9) Plastic bucket for use as a waste receptacle

(10) Microwell holder (Neogen item #9402)

(11) Timer (Neogen item #9426)

(12) Waterproof marker

(13) Wash bottle (Neogen item #9400)

(14) 2 reagent boats for 12-channel pipettor (Neogen item #9435)

(15) Distilled or deionized water

REVISION HISTORY

Revision 0 (03/17/2015)